# A meta-analysis of integrated multi-trophic aquaculture

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**A meta-analysis of integrated multi-trophic aquaculture: Extractive species growth is most successful within close proximity to open-water fish farms**

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**Running title**

Extractive species in open-water IMTA

**Abstract**

Fish farming in open water releases dissolved and particulate waste (inorganic and organic) into the surrounding marine environment. To reduce this environmental impact, commercial extractive species can be grown alongside to utilise and reduce this waste, a technique known as integrated multi-tropic aquaculture (IMTA). Information is lacking on whether: 1) IMTA is generally successful with respect to extractive species growth responses; 2) at what spatial scale they can be cultivated from fish cage nutrient sources. Focussing on bivalves and macroalgae as extractive species, this study uses a meta-analysis approach to summarise and conclude peer reviewed data on IMTA to address these information gaps. We show that there are clear benefits to integrating bivalves and macroalgae with fish farms. Bivalves grown within, and relatively near, fish cages (0 m and 1-60 m distance categories respectively) showed significantly higher biomass production relative to controls compared to those grown at larger spatial scales (61+ m). However, biomass production of macroalgae was significantly higher than controls only within close proximity to fish cages (0 m). This information shows increased extractive species production is generally greatest at relatively small spatial scales. It also highlights the need for more site specific information (e.g. seawater parameters, hydrodynamics, food supply, farm capacity) in future studies. The allocation of control sites and locating these at suitable distances (>1 km) from fish farm effluent sources to avoid fish farm nutrient contamination is also recommended.

**Keywords:** Bivalves; extractive species; fish farm; integrated multi-trophic aquaculture (IMTA); macroalgae; sustainable aquaculture.

## 1. Introduction

Large-scale increases in the intensive mariculture of high-value carnivorous organisms can often result in a number of environmental and sustainability problems (Naylor et al, 2000; Valiela et al, 2001; Naylor et al, 2005; Cabello, 2006; Bergqvist & Gunnarsson, 2013). Consequently, this has contributed towards a generally negative public perception of aquaculture, particularly in Western nations (Mazur & Curtis, 2008) which could restrict the potential for future growth in this much needed sector (White et al, 2004). A particular concern is the release of allochthonous nutrients into the surrounding water column from the rearing of carnivorous fish (e.g. Salmonids) in floating sea cages (open-water farming). Nutrients are released as both particulate (organic) waste (uneaten feed pellets and fish faeces) and as dissolved (inorganic) nutrients as a result of nutrient leaching from particulate waste and fish excretory products (Olsen & Olsen, 2008; Wang et al, 2012). Nutrient loading due to fish farming is considerable (Wang et al, 2012) and can negatively impact the benthic environment due to smothering and increased organic enrichment, leading to alterations in sediment chemistry with knock-on effects on benthic biodiversity (Giles, 2008; Olsen & Olsen, 2008; Hargrave, 2010). Many attempts to reduce nutrient loading surrounding fish farms have been made (e.g. improving the digestibility of fish feeds, computerized feed-management systems), however such technological improvements have not yet eliminated the problem of nutrient pollution associated with fish farming (Islam, 2005; Wang et al, 2012).

One solution to reducing the environmental impact of fish farming is the use of integrated multi-trophic aquaculture (IMTA). IMTA can be used to potentially recycle these nutrients by cultivating additional commercially relevant organisms. These ‘extractive species’ are able to intercept and assimilate aquaculture derived waste (both organic and inorganic) when cultivated alongside fed fish species (Edwards et al, 1988; Chopin et al, 2001; Neori et al, 2004; Troell et al, 2009). This IMTA approach could therefore potentially bio-mitigate the negative environmental impacts of aquaculture whilst simultaneously providing a secondary marketable product for the farmer with possible economical benefit and improved public perception (Chopin et al, 2001; Troell et al, 2003; Ridler et al, 2007). In practice IMTA can take the form of a large variety of systems particularly in Asia (e.g. temporal integration of rice and shrimp or the polyculture of shrimp, fish and crabs in brackish ponds; Troell, 2009) however the majority of Western IMTA operations are land-based, recirculating systems successfully rearing crops of finfish, macroalgae and macroalgivores (e.g. Neori et al, 1996). The majority of general Western aquaculture activities are carried out at sea but at present, there are relatively few commercial examples of open-water IMTA systems (Barrington et al, 2009).

A variety of organisms (e.g. echinoderms or crustaceans; Cook & Kelly, 2007; Barrington et al., 2009; Nelson et al, 2012) have been included as part of open-water IMTA trials, however the most commonly cultivated extractive groups are bivalves and macroalgae. In contrast to echinoderms, bivalves and macroalgae are cultivated down current of fish cages, allowing natural water currents to move farm nutrient waste towards these suspended extractive species. The groups can be divided into organic (bivalves) and inorganic (macroalgae) extractive species based on whether the group in question utilises the organic or inorganic nutrients released from fish farms. Suspension feeding bivalves are generalist consumers, able to ingest a variety of particle types and sizes, therefore particulate fish waste could provide an additional food source for bivalves (Jones & Iwama, 1991; Troell et al, 2003). Laboratory and field studies utilizing stable isotopes and fatty acids as biomarkers have confirmed that bivalves (*Mytilus edulis, Mytilus galloprovincialis, Perna viridis*) are able to capture and assimilate fish farm derived organic waste (Lefebvre et al, 2000; Mazzola & Sara, 2001; Gao et al, 2006; Reid et al, 2010; Redmond et al, 2010; MacDonald et al, 2011). Similarly, Pacific oysters (*Crassostrea gigas*) have demonstrated high growth rates (0.7% $day^{-1}$) when used as biofilters in land-based IMTA systems (Shpigel, 2005). Although mathematical models have suggested that the capacity of bivalves to assimilate farm derived waste may be limited in an open-water context (Cranford et al, 2013), the high food supply environment surrounding fish farms potentially provides an opportunity for increased bivalve growth (Page & Hubbard, 1987; Brown & Hartwick, 1988).

Macroalgal species chosen for inclusion within open-water IMTA operations are typically those with value either as a foodstuff (e.g. *Saccharina latissima*), for industrial applications such as agar extraction (e.g. *Gracilaria spp*), or the cosmetics market (e.g. beauty spas and products). Most are capable of utilising ammonium cations ($NH_4^+$) which is the primary nitrogen species emitted by fish farms (Hanisak, 1983; Chen et al, 2003; Fernandez-Jover et al, 2007). Nitrogen availability is often a major constraint limiting macroalgal growth, particularly in temperate but also some tropical regions (Lobban & Harrison, 1996; Larned, 1998). Therefore, in areas where macroalgal growth is nitrogen limited (e.g. northern temperate regions) greater availability of inorganic nitrogen found within the vicinity of fish farms could result in increased macroalgal growth rates (Chopin et al, 2001; Neori et al, 2004). Ammonium levels immediately surrounding fish farms have been observed to be below the saturation threshold for macroalgae such as *S. latissima* (Ahn et al, 1998) and *Gracilaria vermiculophylla* (Abreu et al, 2011a) suggesting that macroalgae are capable of fully exploiting these available nutrients (Sanderson et al, 2012; Handå et al, 2013). Such uptake has been evidenced within controlled land-based IMTA systems, with 72 % of nitrogen removed concurrent with increased macroalgal growth (e.g. Neori et al, 2000; Chopin et al, 2001; Matos et al, 2006; Abreu et al, 2011b). Based on this evidence, it can be expected that macroalgal species will show high growth rates in the vicinity of fish farm structures releasing high inorganic nutrient loads.

Land-based IMTA systems are mostly closed loop systems thus allowing control of nutrient rich waste (Chopin et al, 2001). In contrast, open-water IMTA lacks this fine control with the dilution of waste occurring by natural seawater movement (e.g. currents). These systems are however generally sheltered within fjordic systems (e.g. Scottish sea lochs) and are likely to have regular current patterns (Navas et al, 2011) leading to the general assumption that the organic and inorganic nutrients will progressively disperse as distance increases from the farm. This increased dilution with distance from farm effect may severely affect the ability of extractive species to intercept nutrient rich waste, thus raising concerns over the effectiveness of using IMTA within an open-water context (Cranford et al, 2013). There is therefore a knowledge gap on the spatial scale at which extractive species can be located in order to assimilate waste and increase profitability.

This study will focus only on extractive species growth at different spatial scales to determine whether IMTA can be regarded as worthwhile. Individual trials investigating the cultivation of macroalgae as an extractive species show some indication of positive results whereas those using bivalves as extractive species are less clear. To date this information has not been collated in an informative manner. An overview to determine the effectiveness of open-water IMTA in the context of the production of extractive species is therefore required. Such an overview could help stakeholders determine whether IMTA practices are worth adopting, as well as contributing towards Blue growth (Whitmarsh et al, 2006; DEFRA, 2015).

This study aims to summarise from the available literature whether open-water IMTA results in extractive species growth augmentation. More specifically, it will focus on the growth of bivalves and macroalgae cultivated in the vicinity of open-water fish farms. Growth responses at increasing distances from the fish cage will also be investigated to help determine the best location to place the extractive species in relation to the farm, information which could be useful for IMTA implementation. We hypothesize that extractive species will show increased growth (relative to controls) when cultured alongside fish farms with growth augmentation declining as distance increases from the closest fish cage.

## 2. Methods

### *2.1. Data selection*

A comprehensive search of peer reviewed literature was carried out during early 2015 using a keyword search of the Web of Science database. Studies were located using the terms; “bioremediation”, “bivalve”, “growth”, “IMTA”, “integrated aquaculture”, “macroalgae”, “mussel”, “polyculture”, “salmon” and “seaweed” with those studies of direct relevance to the subject matter of this study selected for use. All literature selected compared the growth rate of extractive species (bivalves or macroalgae) cultivated in the vicinity of commercial open-water fish farms with an expressly specified control. Studies which did not include a designated control were excluded from this analysis. All studies were experimental interventions except for Wallace (1980) who measured the growth of naturally occurring fouling mussels on artificial structures both in the vicinity and at a distance from fish cages. Only studies which provided all necessary data (growth parameters, standard deviation values, sample size) were included within this analysis. Data were restricted to the use of cultivated extractive species of potentialcommercial interest (Bivalves: *Crassostrea gigas, Mytilus edulis, Mytilus galloprovincialis, Mytilus planulatus, Ostrea edulis* and *Placopecten magellanicus;* Macroalgae: *Gracilaria chilensis, Palmaria palmata, Sargassum hemiphyllum, Sargassum henslowianum, Saccharina latissima* and *Ulva spp)*. Data were further restricted to those studies which quantified shell length for bivalves and the parameters blade length (cm), biomass production (kg fresh mass $m^{-1}$) and specific growth rate (SGR; % $day^{-1}$) for macroalgae. The latter was averaged across the total length of time for each respective study. Data were extracted from graphical figures within the literature using digitizing software (PlotDigitizer; http://plotdigitizer.sourceforge.net).

Several of the studies included within the meta-analysis contributed more data points than other studies. For example, Navarrete-Mier et al (2010) measured the growth of two extractive species (*O. edulis* and *M. edulis*) at five different distances (0 m, 25 m, 120 m, 300 m & 600 m) from the nearest fish farm thereby contributing ten data points to the meta-analysis. In contrast, measuring the growth of one extractive species (*C. gigas*) at one distance (e.g. Jiang et al, 2013) contributed only a single data point towards the meta-analysis. In this study the difference in growth in extractive species between experimental (IMTA) and control sites for each distance was treated as a separate data point, providing the selection criteria described above were met. Although using multiple observations from a single study can decrease the independence of these data points, it was necessary due to the limited number of studies suitable for inclusion (e.g. Kroker et al, 2010).

*2.2. Data analysis*

All studies used in this meta-analysis compared the growth rate of extractive species grown in the vicinity of open-water fish farms with those of a designated control, therefore standardized mean difference was used as the effect size. Effect size is used to quantify the magnitude of difference between two groups with the difference expressed in standard deviation units (Sullivan & Feinn, 2012). A positive value for effect size indicates the experimental group outperformed the control group, a negative effect size indicates underperformance. An effect size of zero indicates no difference between experimental and control groups. For each data point, standardized mean difference was expressed as Cohens d' which was calculated using Formula 1 (Gurevitch et al, 2001; Lakens, 2013) where $M^E$ and $M^c$ represent mean extractive species size at the end of the experimental period for the experimental and control groups respectively. The use of Cohens d' can give a biased estimate of effect size when sample sizes are small (< 20) or differ between experimental and control groups (Hedges & Olkin, 1985). This was encountered within this analysis, therefore an unbiased corrected effect size (Hedges g') was used in this analysis. Hedges g' was calculated from Cohens d' using Formula 2 where $n_E$ & $n_C$ represent sample size for the experimental and control groups respectively (Gurevitch et al, 2001; Lakens, 2013). Variance in effect size ($V_D$) can be calculated by squaring standard error in effect size ($SE_D$) which is calculated using Formula 3 (Gurevitch et al, 2001; Lakens, 2013), where d is effect size (Hedges g').

**Formula 1:** $\text{Cohens d} = (M^E\text{-}M^c)/SD_{pooled}$

**Formula 2:** $\text{Hedges g'} = \text{Cohens d'} \times [1\text{-}(3/4(n_E+n_C)\text{-}9)]$

**Formula 3:** $SE_D = \sqrt{([(n_E+n_C)/n_E n_C]+[d^2/2(n_E+n_C)])}$

The studies used in this analysis varied due to interspecific differences between species (e.g. growth rate) and variation in site-specific conditions (e.g. temperature, salinity & chlorophyll-*a* levels). Therefore, a random-effects model was used to calculate the weighted mean effect size. Random-effects models account for two sources of sampling error; within-study variance and between-study variance. Within study variance is given by $V_D$ (see above). Between study variance (r) was calculated by subtracting the degrees of freedom (n-1) from total variance and then dividing by a scaling factor, using equations given by Borenstein et al, 2007. Total variance ($V_D$*) for each data point was calculated by adding together $V_D$ and r. The reciprocal of $V_D$*, $w_i$ was used to determine the weighting each data point carried within the combined effect.

For each study a weighted mean effect size (T) was calculated. All data points from each study were combined using Formula 4 (Borenstein et al, 2007) where $T_i$ is effect size (Hedges g'). The standard error of mean effect size ($SE_T$) was calculated using Formula 5. The significance of weighted mean effect size was assessed by constructing 95% bootstrapped confidence intervals around weighted mean effect size using equations given by Borenstein et al (2007). If 95% confidence intervals do not cross zero, weighted mean effect size can be considered significant (Borenstein et al, 2007). Forest plots were constructed to show the results graphically (weighted mean effect size ± 95% confidence intervals). Studies which contributed a single data point (e.g. Sara et al, 2009) were presented simply as Hedges g' for graphical representation of effect size. The total weighted mean effect size (T*) was then calculated by combining all of these weighted data points from all studies for bivalves and macroalgae (Formula's 4 & 5). All calculations were performed on Microsoft Excel 2016 (version 16.0) using the framework provided by Neyeloff et al (2012) as a guide.

**Formula 4:** $T = \Sigma (w_i T_i) / \Sigma w_i$

**Formula 5:** $SE_T = \sqrt{(1/w_i)}$

*2.3 Distance subgroup analysis*

To determine the effect of distance on the growth of extractive species, each data point was categorized into a subgroup. Bivalve data points were categorized into four distance categories between the bivalves and the nearest stocked fish cage; 1) 0 m, 2) 1-60 m, 3) 61-299 m and 4) 300+ m. "0 m" indicates the bivalves were located inside, suspended underneath or "immediately adjacent" to the fish cage. Previous studies have reported that 99

% of particles originating from fish farms will settle within 60 m (Coyne et al, 1994; Giles, 2008), therefore a distance category of "1-60 m" was also used in this analysis. Not all studies reported an explicit distance between bivalves and fish cages, studies which stated experimental bivalves were located "adjacent" to fish cages were presumed to be between 1 and 60 m of fish cages and thus were included within this distance category. Bivalves taken from floats supporting fish farms (Wallace, 1980) were also included within the "1-60 m" category. To maximise categorical balance, the threshold between the 3$^{rd}$ and 4$^{th}$ distance categories was set at an arbitrary value of 300 m.

Macroalgae data points were categorized into three distance categories; 1) 0 m, 2) 1-60 m and 3) 61+ m. "0 m" indicates macroalgae were cultivated "within" or "attached" to the fish farm. Major nutrient enhancement is found within 60 m of fish farms (Sanderson et al, 2008), therefore, similar to bivalves (described above) the next category was set as "1-60 m". Not all studies reported an explicit distance between macroalgae and fish cages. Studies which stated that macroalgae were located "adjacent" to fish cages were presumed to be between 1 and 60 m of fish cages and thus were included within this distance category. As only five data points were made at distances exceeding 60 m, to maximise categorical balance the final category was therefore set as "61+ m". A weighted mean effect size with 95% confidence intervals was then constructed for each individual study and subgroup in addition to total weighted mean effect size using the methods described above.

### *2.4. Sensitivity*

To test the robustness of our findings, a sensitivity analysis was performed using the method employed by Kroeker et al (2010). To summarise, those studies with the largest effect size (regardless of distance) were systematically removed from the meta-analysis, which was then re-run to determine what effect removal had on the meta-analysis outcome. This step was then repeated with effect sizes of decreasing magnitude to determine how many studies needed to be removed to change the significance of the overall result. Similarly, if any study contributed five or more data points to the meta-analysis then this study was removed and the meta-analysis re-ran.

## 3. Results

### *3.1. Bivalves*

Twelve studies were found which compared the growth of bivalves cultivated in the vicinity of fish farms with designated controls. From these 12 studies, 43 data points were extracted and incorporated within the meta-analysis. The analysis showed that IMTA had an overall significantly positive effect on the growth rate of bivalves, as indicated by total weighted mean effect size ($T^*$; Figure. 1). However, the growth augmentation bivalves experienced in open-water ITMA systems varied according to distance from the closest fish farm. Bivalves within the 0 m and 1-60 m subgroups showed significantly higher growth than controls whereas bivalves grown at further distance points (61 – 299 m and 300+ m) grew at a similar rate to control bivalves (Figure. 2).

The stepwise removal of the fifteen largest effect sizes and the removal of the three studies which contributed five or more data points (Jones & Iwama, 1995; Navarrete-Mier et al, 2010; Lander et al, 2012) did not alter the significance of either total weighted mean effect size or weighted mean effect size for each distance category. The sensitivity analysis therefore indicates that the findings of this meta-analysis are robust.

### *3.2. Macroalgae*

Eight studies were found which compared the growth of macroalgae cultivated in the vicinity of fish farms with designated controls. From these eight studies, 24 data points were extracted and incorporated within the meta-analysis. The analysis showed that IMTA had an overall significantly positive effect on the growth of macroalgae, as indicated by total weighted mean effect size ($T^*$; Figure. 3). However, the growth augmentation macroalgae experienced in open-water ITMA systems varied according to distance from the closest fish farm (Figure. 4). Macroalgae within the 0 m subgroup grew significantly faster than controls whereas macroalgae grown further in distance (1-60 m and 61+ m) grew at a similar rate to control macroalgae.

A single study (Sanderson et al, 2012) contributed five or more data points to this meta-analysis. The removal of Sanderson et al (2012) did not alter the significance of total weighted mean effect size. However, the removal of the five largest effect sizes from the database used to calculate total weighted mean effect size altered the significance of total weighted mean effect size (significant to non-significant). Stepwise removal of high magnitude data points from the subgroup analysis did not alter the significance of any findings. As the removal of high magnitude data points altered the significance of total weighted mean effect size, the findings for macroalgae are less robust than for bivalves.

## 4. Discussion

The growth of extractive species (bivalves and macroalgae) was significantly greater than controls when integrated with open-water fish farms. Macroalgae cultivated within fish farms (0 m) performed significantly better than those at increasing distances. Bivalves cultivated within (0 m) and near (1-60 m) fish farms showed significantly greater growth than those located at further distances (61+ m). Extractive species therefore show best growth performances when located within close proximity to fish farms. Macroalgae generated higher growth rates when integrated within the farm but bivalves showed a larger spatial scale of up to 60 m distance for highest growth performances. Overall, these results demonstrate that IMTA is effective with respect to extractive species augmentation which could help farmers generate a profit, particularly if compared to monospecific farms. Although these results lend support to the implementation of open-water IMTA systems, the meta-analyses showed high variation. Such variation can be attributed to many factors which we will now discuss.

### *4.1. Sources of variation*

#### *4.1.1. Seasonality and nutrient / food supply*

The growth enhancement of macroalgae cultivated as part of open-water IMTA systems can be attributed to a fertilisation effect due to increased nutrient levels found within fish farms. Further evidence for the utilisation of farm derived inorganic nutrients is provided by the increased nitrogen content of macroalgae cultivated 10 m from fish cages and by the enrichment of *S. latissima* in a nitrogen isotope ($\delta^{15}$) typical of fish effluent (Troell et al, 1997; Sanderson et al, 2012). Many factors can influence nutrient levels surrounding fish farms including feeding regime, hydrodynamics (Sanderson et al, 2008) and ambient nutrient levels. Macroalgal growth is often limited during the summer due to low ambient nutrient levels (Lobban & Harrison, 1996), it can therefore be expected that IMTA macroalgae will experience the greatest growth enhancement during summer months due to the availability of dissolved nutrients released by nearby fish farms (Chopin et al, 2001; Neori et al, 2004). Studies included within this meta-analysis support this theory with Abreu et al (2009) observing greatest growth rates of *G. chilensis* during the summer. Similarly, Handå et al

(2013) found the growth enhancement of IMTA macroalgae to be most pronounced in summer and Wang et al (2014) observed the growth increase of *S. latissima* to occur at a time when dissolved nitrogen levels were at their lowest. In contrast, Halling et al (2005) found no significant difference in macroalgal biomass production between IMTA and control sites. Although ambient nutrient levels were not expressively measured by Halling et al (2005), the findings of this study may have been influenced by seasonality given that the study occurred through the austral winter (when ambient nutrients may not limit macroalgal growth). Troell et al (1997) who cultivated the same species (*G. chilensis*) at the same site but instead during summer months reported a 40% increase in integrated macroalgae production when compared to controls. These examples provide strong evidence that the seasonal timing of IMTA trials may influence the results. It is therefore likely that macroalgae cultivated by Halling et al (2005) may not have received the full benefits of integration due to their selected growth period.

Other seasonality related factors may also influence the growth benefit macroalgae experience when integrated with open-water fish farms. Nutrient emissions from open-water fish farms vary, but generally increase during the course of the grow-out cycle (typically two years) peaking (up to four-fold) in late summer of the second year when fish feeding levels are highest (Strain & Hargrave, 2005; Reid et al, 2013a). Commercial macroalgal harvest would have to occur during early to mid-summer as macroalgae begins to degenerate in late summer (Lobban & Harrison, 1996), therefore peak nutrient emissions from fish farms would not be available to IMTA extractive macroalgae. Consequently, for a large period of the year (particularly during the first year of fish rearing) macroalgae would not be exposed to substantially elevated nutrient levels. How macroalgal growth augmentation varies during the typical two year grow-out cycle has yet to be assessed. Such information is required because commercial IMTA ventures which integrate macroalgae will need to account for variations in farm derived nutrient emissions e.g. by scaling back macroalgal cultivation during the first year of fish growth.

Food availability for bivalves varies due to a variety of factors (e.g. light, nutrient levels) that undergo regular spatial and temporal fluctuations (Page & Hubbard, 1987; Navarro & Thompson, 1995; Litchman, 1998; Cranford & Hill, 1999). At mid to high latitudes phytoplankton levels are at their seasonal minima throughout the winter, therefore natural populations of bivalves often show minimal growth during this period of food limitation (Malouf & Breese, 1977; Hilbish, 1986). Wallace (1980) suggested that the faster growth rates of *M. edulis* observed at fish farms was likely due to mussels receiving a continuous supply of farm derived waste throughout the winter, thus facilitating year round growth. Similar conclusions were made by Lander et al (2012) who found that the growth advantage gained by *M. edulis* in the vicinity of fish farms was most pronounced in the autumn and winter months. The importance of seasonal timing could explain why some studies found bivalves showed no significant growth enhancement through IMTA. Part of the study carried out by Cheshuk et al (2003) was conducted on an empty farm (no fish present) during the austral winter period (3 ½ months from June to September). During this time these mussels (*M. planulatus*) were not exposed to farm derived waste. Chlorophyll-*a* measurements showed that natural food availability was at its lowest during this time, thus leading to the low growth rates reported.

When ambient particle concentration, often described as total particulate matter (TPM), exceed a certain threshold (e.g. 5.0 mg TPM $l^{-1}$ for *M. edulis)* then a significant proportion of ingested particles are not digested but instead are rejected as pseudofaeces (Widdows et al, 1979). Saturation of mussel feeding due to high ambient TPM was suggested by Cheshuk et al (2003) as a possible mechanism for why only modest enhancement of *M. planulatus* growth was observed. The use of bivalves as extractive species in open-water IMTA fish farms is dependent on bivalves directly consuming particulate fish waste. The validity of such systems may therefore be compromised if ambient particle concentrations surrounding fish farms were consistently higher than the pseudofaeces threshold. Therefore, bivalve growth enhancement may likely be achieved in IMTA systems located in areas with seasonally or consistently low ambient seston levels (Troell & Norberg, 1998). In oligotrophic waters, farm derived nutrients could also stimulate local phytoplankton production thereby increasing the food supply for secondary consumers e.g. bivalves (Sara et al, 2009). The relationship between bivalve-fish IMTA and variations in local food supply is complex and will require more focus in future IMTA studies. Quantification of the assimilation of farm derived waste at varying particle concentrations would assist in elucidating the relationship between the outcome of open-water IMTA and ambient seston levels.

#### *4.1.2. Hydrodynamics*

Models indicate that bivalves are best able to capture particulate fish waste when cultivated in areas with slow (< 0.05 m $s^{-1}$) current speeds (Troell & Norberg, 1998; Cranford et al, 2013). Studies included within this meta-analysis measuring faster current speeds of up to 0.11 m $s^{-1}$ (e.g. Navarette-Mier et al, 2010) found no evidence for bivalve growth augmentation. Particle capture efficiency is dependent on the amount of time available to filter particles from the surrounding water column which is dependent on current speed (fast current speeds equal less time to extract food particles and *vice versa*). IMTA bivalves cultivated in areas where currents do not regularly exceed 0.05 m $s^{-1}$ can therefore be expected to show greater growth enhancement when integrated with open-water fish farms (Troell & Norberg, 1998; Cranford et al, 2013).

Nutrient dispersal surrounding open-water fish farms is influenced by hydrodynamics, subsurface geographical features and the structure of the fish cage (Sanderson et al, 2008). Understanding dispersal patterns and how they change over time is a complex task requiring extensive field work and advanced modelling (Olsen et al, 2008). An understanding of nutrient emissions from open-water fish farms (also referred to as volumetric loading) would be of importance for commercial IMTA ventures, because it would allow farmers to obtain the maximum growth benefit for their crop through optimum placement of extractive species. Optimum placement is likely to be highly site-specific therefore this meta-analysis cannot provide detailed information on how to organise a commercial IMTA farm besides showing the general distances at which extractive species can be cultivated.

#### *4.1.3. Species specific responses*

Species specific differences in fish faecal properties, extractive species optimal growth, assimilation and feeding mechanisms and patterns will have contributed towards our results. Bivalve growth rates differ intrinsically between species, as shown by comparative studies (Epifanio, 1979; Laing et al, 1987; Cardoso et al, 2006). Intra-specific variation could thus have contributed to the varying growth responses seen in IMTA bivalves as seen by Rensel et al (2011). However, given the small number of studies suitable for inclusion in this meta-analysis, the data were not used to determine the species specific level of contribution, of farmed fish or extractive species, to the effect sizes reported in this study. More open-water IMTA intervention studies on a range of farmed fish and extractive species types would help to determine which species provide the biggest influence on IMTA responses. Despite this variation, increased extractive species growth found during this study demonstrates that increased extractive species production is generally achievable across a range of species.

#### *4.1.4. Control site selection*

Macroalgae have demonstrated increased production at distances as great as 800 m from fish farm effluent sources (Abreu et al, 2009). In some cases, control sites have been located within this range and demonstrate pronounced biomass production (Halling et al, 2005). It is therefore likely that these sites have been located within the dispersal range of fish farm effluents and therefore could mask potential farm specific responses. To avoid downstream nutrient contamination of controls in future studies, we recommend that control site location considers the hydrodynamics of the area and suitably high distances (e.g. > 1 km (8 km as used by Abreu et al (2009)) from fish cages. Furthermore, the selection criteria for this meta-analysis outlined that literature which did not include a designated control were excluded from the analysis, and as a consequence several studies were not used in the analysis. We therefore recommend the use of well-placed controls in future intervention experiments to help increase the body of evidence around IMTA responses.

*4.1.5. Site specific information*

The description of site specific conditions was variable or sometimes absent in the literature (e.g. chlorophyll-*a* concentration, mean current speed). This information is required to understand what effects (if any) site-specific conditions have on the capacity of extractive species to capture and assimilate fish waste. This information is also valuable to farmers by allowing identification of localities where commercial IMTA ventures are most likely to succeed. Based from our experience in this meta-analysis we recommend that future studies consider the following information for study areas: water temperature and salinity, mean and maximum current speeds and their direction, chlorophyll-*a* concentration, particulate organic matter and TPM concentrations. Additionally, details on fish-farm size and feeding protocols would be beneficial towards understanding the effect of distance between fish cages and extractive species.

*4.2. Meta-analysis limitations*

One of the limitations with meta-analysis is the reliance on publicly available data which could create a bias on the reported effect sizes. This is a possibility for the data used within this meta-analysis because the majority (80 %) of the macroalgal growth responses included in this meta-analysis demonstrated strong evidence for increased macroalgal growth. Half of the bivalve studies used in this meta-analysis reported positive growth responses relative to controls. It is possible that data has not been made publicly available from studies which were unsuccessful in extractive augmentation within IMTA. Such information will be critical for future meta-analytical summaries of IMTA implementation as more literature is released in this field of research. It is therefore highly recommended that researchers and journals encourage publicising data which demonstrates unsuccessful extractive augmentation within IMTA to prevent possible future bias.

The initial literature search identified 14 studies which cultivated macroalgae in the vicinity of open-water fish farms. However, six of these studies were excluded from subsequent analysis due to the lack of an expressly specified control or a lack of reporting of data (e.g. sample size) required for the meta-analysis. Therefore, only eight macroalgal studies (containing 24 data points) were included within this analysis, compared to 12 bivalve studies (containing 43 data points). Given that significant bivalve growth enhancement was found within the 1-60 m distance category despite the generally rapid settling velocity of particulate fish waste (Law et al, 2014), the lack of significant macroalgal growth enhancement at distances greater than 0 m could be deemed surprising. The paucity of suitable macroalgal studies could be a potential causative factor behind the lack of significant macroalgal growth enhancement at distances greater than 0 m. This therefore emphasises the recommendation for future IMTA studies to include suitable controls within experimental designs as it is only with reference to controls that the presence (or lack of) growth enhancement can be determined.

### *4.3. Logistics of extractive species in IMTA*

As the dilution of particulate fish waste increases with distance from fish cages (Doglioli et al, 2004), bivalves cultivated within fish cages themselves (0 m) will be exposed to higher concentrations of particulate waste (and thus increased food availability) than those at greater distances. Therefore, greater growth augmentation for bivalves and macroalgae within the 0 m subgroup is predictable. However, significantly increased bivalve growth was also observed within a larger spatial scale, in this case up to 60 m. Although significant macroalgal growth augmentation was only found for the 0 m subgroup, individual studies have found significantly increased macroalgal production at distances of up to 800 m from fish farms (Abreu et al, 2009). Such findings are encouraging as farmers are unlikely to adopt IMTA practices if the installation of extractive species interferes with the day to day operations of the fish farm (the major cash crop) e.g. restricting access to fish cages or impeding water flow (thus reducing oxygen supply to fish). Therefore, extractive species must be appropriately located (e.g. not inside a fish cage). The results of this meta-analysis indicate that spatial constraints may not represent an impediment to widespread open-water IMTA. To maximise farm waste recapture (as well as biomass production), bivalves should be cultivated close to fish cages due to the rapid settling velocity of particulate fish waste (Law et al, 2014). However, care should be taken in locating the macroalgal component of open-water IMTA farms as excess particulate fish waste could potentially settle on macroalgal fronds thus blocking light and restricting growth.

The evidence presented in this study shows that by adopting IMTA practices, economic advantages could be gained by farmers (though increased production of extractive species). By providing secondary marketable crops, IMTA farms exhibit a greater degree of economic diversification compared to monoculture operations. Diversification represents a form of insurance, as a marketable product will still be produced in the event of disease outbreaks or infrastructure damage (e.g. net failure). Profitable markets presently exist for the sale of bivalves (Lucas & Southgate, 2012) and the farmed seaweed market is likely to grow in Western nations given the increasing popularity of seaweed consumption (Brownlee et al, 2012). Much of the infrastructure and equipment for IMTA (e.g. rope lines, boats, buoys) will already be present on fish farms, therefore the start-up costs of IMTA in farms is likely to be low (Lander et al, 2012). However, labour costs can be expected to increase on IMTA farms as extra work hours will be required for the maintenance and harvesting of extractive species (Holdt & Edwards, 2014). A model based on a Canadian farm estimates that net present value (NPV) is increased by 24% when mussels and seaweed are grown alongside Atlantic salmon (Ridler et al, 2007) however more transparent models containing greater detail (e.g. proportions of extractive species) would be of use to farmers in determining the economies of integration. Surveys have shown a positive attitude towards IMTA amongst the general public indicating that consumer acceptance will not be a barrier to IMTA expansion, with 50% of participants willing to pay 10% extra for IMTA labelled products (Barrington et al, 2010). Therefore, a system of eco-labelling may allow IMTA farmers to charge a higher price for their products and thus keep IMTA farms profitable in the face of falling fish prices (Whitmarsh et al, 2006) or competition with larger firms (Ridler et al, 2007). The profitability of IMTA farms may also be improved if coastal management systems legally oblige operations to pay for the environmental cost of their activities via discharge taxes ("user pays" concept; Troell et al, 2003).

However, before IMTA becomes more widely implemented there are a number of mitigation and biosecurity issues regarding commercial IMTA that need to be satisfactorily resolved. While bivalve integration has shown generally positive growth responses in this meta-analysis, the net organic loading from bivalves (released as faeces) combined with the fish farm may still have a negative impact on the underlying benthos. It has been recommended by the Fisheries and Oceans Canada (DFO) Science Advisory Schedule (DFO, 2013) to use extractive deposit feeding species (e.g. sea urchins, sea cucumbers and polychaetes) located underneath the suspended bivalve extractive species to consume these heavy organic solids (Cranford et al., 2013; DFO, 2013; Reid et al., 2013b). The implementation of adding another trophic level into IMTA will require structural considerations relating to the fish farm (e.g. oxygen supply via seawater flow and efficient connection between trophic levels; DFO, 2013).

Previous work has found that IMTA bivalves grown in water of sufficient depth are highly unlikely to act as reservoirs for fish pathogens such as infectious salmon anaemia virus (ISAV) or *Vibrio anguillarum* and may assist in the control of drug-resistant pathogens and parasites such as the sea louse *Lepeoptheirus salmonis* (Mortensen, 1993; Skar & Mortensen, 2007; Molloy et al, 2011; Pietrak et al, 2012; Molloy et al, 2014). Haya et al (2004) similarly found that extractive species (*M. edulis* and *S. latissima)* cultured in the vicinity of a *S. salar* farm did not accumulate hazardous therapeutants or contaminants (e.g. heavy metals) above background levels. To facilitate the spread of open-water IMTA further work regarding bioaccumulation within extractive species and potential disease transfer within farms requires consideration to dispel any concerns farmers or regulatory bodies may have regarding IMTA. If commercial IMTA is to become widespread, legislation governing aquaculture operations may have to be reformed so that policy recommendations (e.g. minimum distances between mussel and fish farms) do not act as barriers to commercial IMTA (Alexander, 2015).

### *4.4. Conclusions and future research considerations*

This study demonstrates that; 1) extractive species cultivated in the vicinity of open-water fish farms experience a growth benefit due to integration and 2) close proximity of extractive species to the farm (0 m for macroalgae and 0-60 m for bivalves) can increase performance and therefore possibly profit but there appears to be some spatial flexibility around this if logistical constraints require it. Even though the extent at which nutrient extraction is carried out is still quantitatively unknown, spatially extensive locations of extractive species are known to significantly reduce organic loading around fish cages (Reid et al, 2013a; Holdt & Edwards, 2014).

Future study recommendations include: 1) allocating control sites and locating these at suitable distances (>1 km to 8km) from fish farm effluent sources to avoid fish farm nutrient contamination; 2) including site details such as seawater parameters (e.g. temperature, salinity), hydrodynamics (current speeds and direction), food supply (chlorophyll-*a*, particulate organic matter and total particle matter concentrations), farm capacity (farm size and feeding protocols); and 3) determining the extent to which spatially extensive extractive species cultivation mitigates nutrient discharge from open-water fish farms (including consideration of the organic loading from the bivalve component of IMTA farms).

Open-water IMTA is still in development and further research can be expected to improve IMTA methodologies (e.g. optimum placings for extractive species and a wider range of commercial extractive species) leading towards more sustainable IMTA systems. Although complete (100%) nutrient sequestration is not practically feasible, future IMTA efforts should be encouraged given the environmental and economic merits of integration.

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**Figure legends**

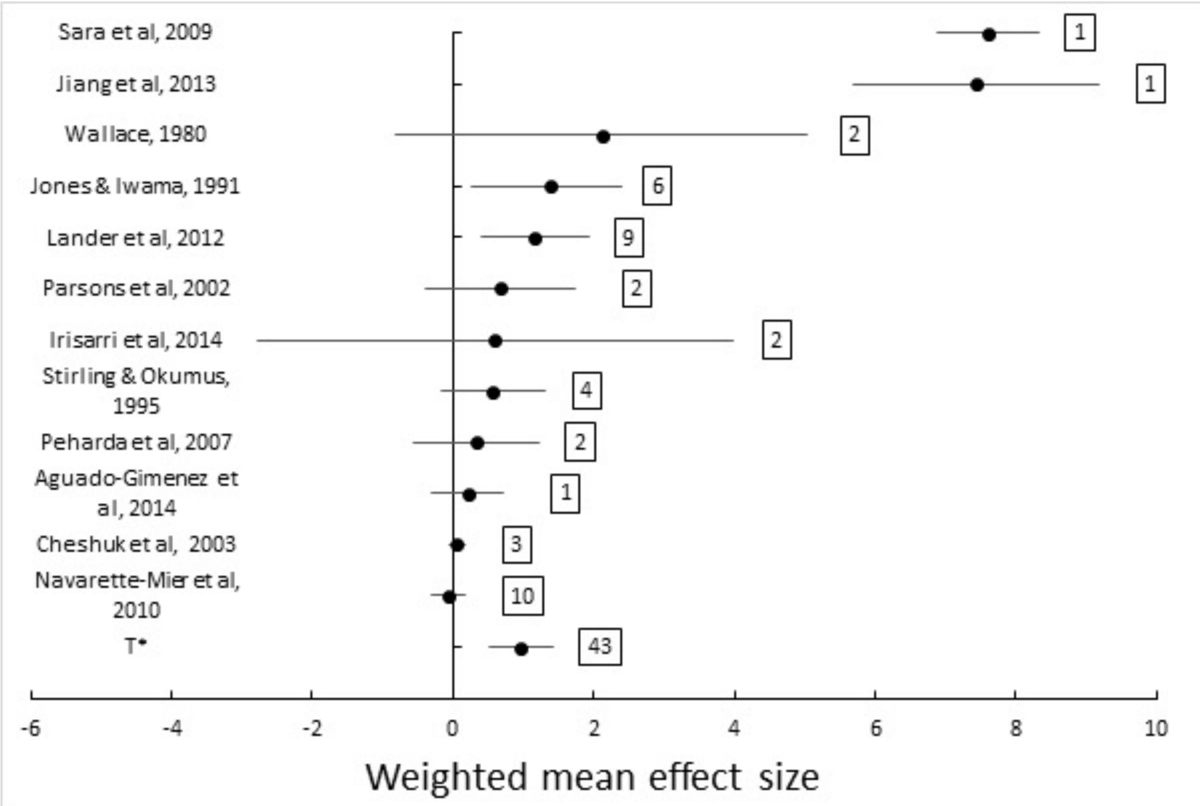


**Figure 1.** Bivalve growth relative to controls (weighted mean effect size ± 95% confidence intervals) when integrated with open-water fish farms presented for individual studies and for all studies collated (total weighted mean effect size (T*)). Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.

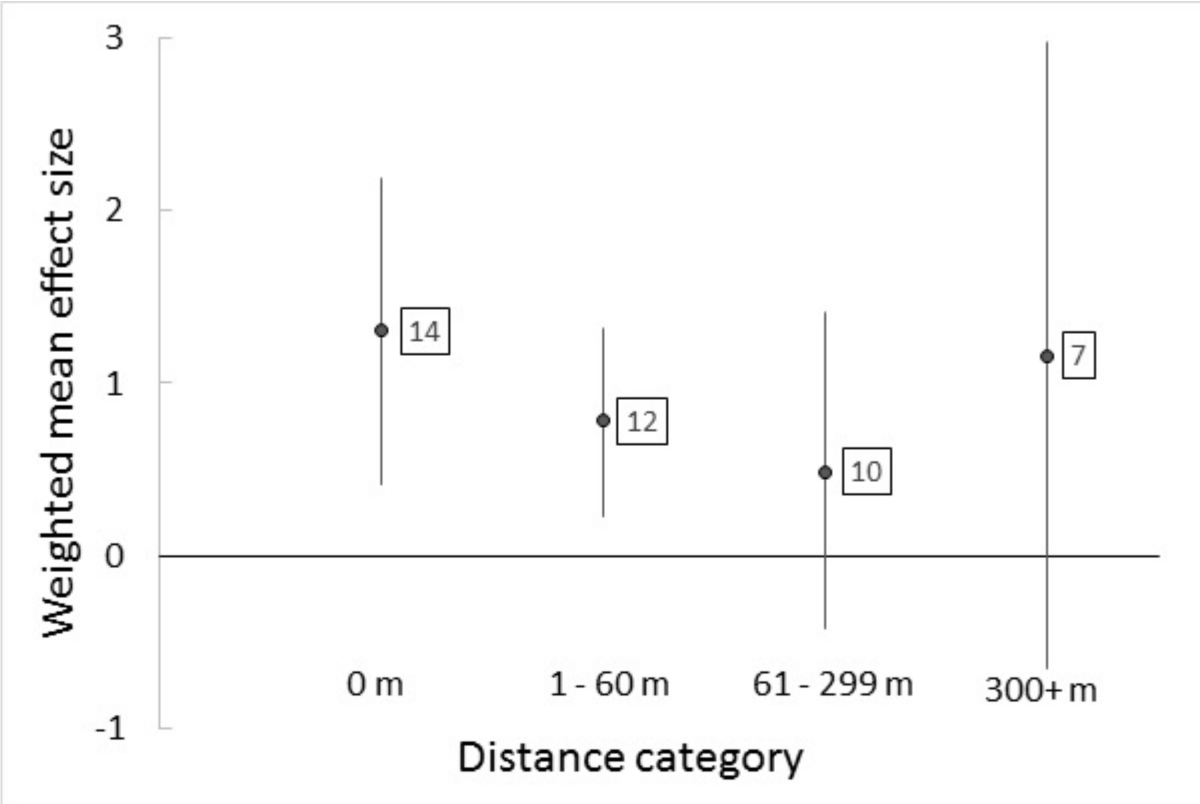


**Figure 2.** Weighted mean effect size (± 95% confidence intervals) for bivalves cultivated at varying distances from open-water fish farms. Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.

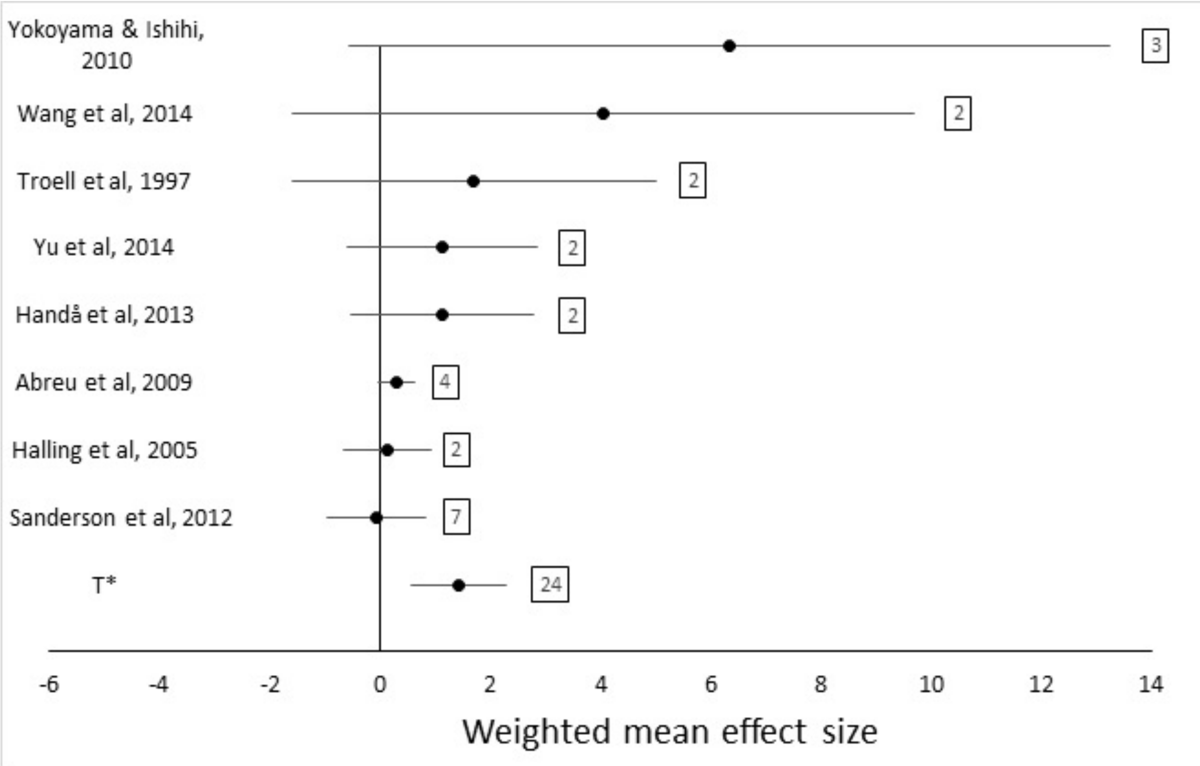


**Figure 3.** Macroalgal growth relative to controls (weighted mean effect size ± 95% confidence intervals) when integrated with open-water fish farms presented for individual studies and for all studies collated (total weighted mean effect size (T*)). Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.

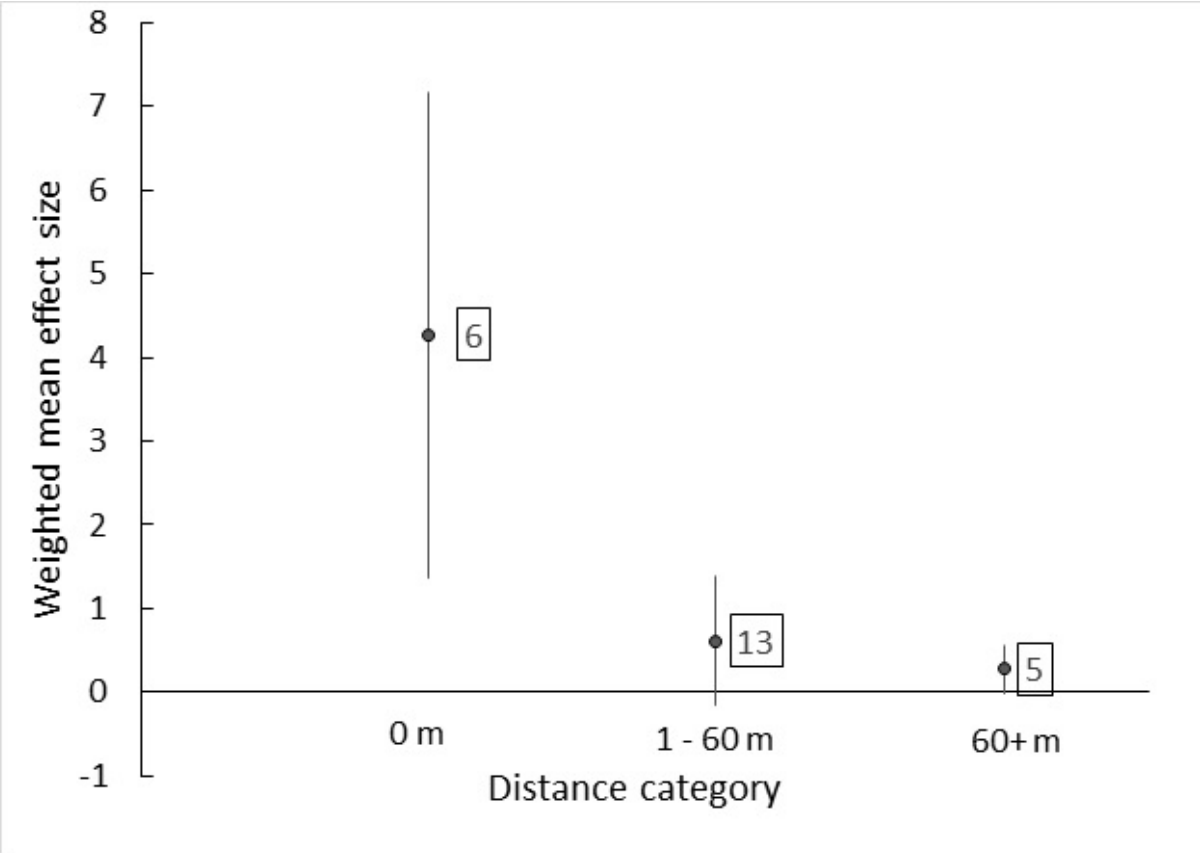


**Figure 4.** Weighted mean effect size (± 95% confidence intervals) for macroalgae cultivated at varying distances from open-water fish farms. Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.